cytokine. This is the first report of the expression of a correctly folded **TGF-beta** superfamily **protein** in a microbial organism. The **protein** is secreted in its correctly folded dimeric form at milligram per litre quantities, which are significantly higher than we have been able to achieve using mammalian expression systems. Purification schemes are described, and the purified **protein** is immunologically identical to **protein** produced in a mammalian expression system. **Protein** expression was influenced by a number of factors, most significantly by the concentration of methanol used during the induction phase. However, with very high levels of MIC-1 induction, substantial amounts of MIC-1 **monomer** were also secreted.

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=> d his

     (FILE 'HOME' ENTERED AT 14:07:50 ON 30 MAR 2002)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 14:08:04 ON
     30 MAR 2002
L1        330132 S MONOMER
L2         57405 S L1 AND PROTEIN
L3           165 S L2 AND TGF BETA
L4             2 S L3 AND AMINO ACID SUBSTITUTION
L5             2 DUP REMOVE L4 (0 DUPLICATES REMOVED)
L6            23 S L3 AND BMP
L7             1 S L6 AND SUBSTITUTION
L8             0 S L3 AND GROWTH DIFFERENTIATION FACTOR 5
L9             4 S L3 AND GDF
L10            3 DUP REMOVE L9 (1 DUPLICATE REMOVED)

=> s l2 and growth differentiation factor
L11            3 L2 AND GROWTH DIFFERENTIATION FACTOR

=> s l2 and GDFs
L12            3 L2 AND GDFS

=> s l2 and GDF
L13            4 L2 AND GDF

=> dup remove l13
PROCESSING COMPLETED FOR L13
L14            3 DUP REMOVE L13 (1 DUPLICATE REMOVED)

=> d l14 1-3 cbib abs

L14  ANSWER 1 OF 3  EMBASE  COPYRIGHT 2002 ELSEVIER SCI. B.V.
2001122533  EMBASE  The crystal structure of the BMP-2: BMPR-IA complex and the
     generation of BMP-2 antagonists. Nickel J.; Dreyer M.K.; Kirsch T.; Sebald
     W.. Dr. J. Nickel, Physiologische Chemie II, Biozentrum der Universitat
     Wurzburg, Am Hubland, 97074 Wurzburg, Germany. sebald@biozentrum.uni-
     wuerzburg.de. Journal of Bone and Joint Surgery - Series A  83/SUPPL. 1
     (S17-S114)  2001.
     Refs: 30.
     ISSN: 0021-9355. CODEN: JBJSA3. Pub. Country: United States. Language:
     English. Summary Language: English.
AB   Background: Bone morphogenetic **proteins** (BMPs) and growth and
     differentiation factors (**GDFs**) belong to the large transforming
     growth factor-.beta. (TGF-.beta.) superfamily of multifunctional
     cytokines. Signaling of the BMPs requires the binding of the BMP to the
     BMP cell surface receptors BMPR-IA, BMPR-IB, and BMPR-II. Similar to other
     cytokines, members of the TGF-.beta. superfamily exhibit stringent
     specificity in their ligand-receptor interactions, which may be a reason
     for the qualitative and quantitative differences in cellular responses. To
     understand how BMPs and GDFs activate their receptors, it is
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important to determine structure and binding mechanisms of ligand-receptor complexes. We have used BMP-2 as a key representative of the BMPs to identify the epitopes for type I and type II receptor binding by mutational interaction analyses and have solved the crystal structure of a BMP-2:BMPR-IA receptor ectodomain complex. Methods: To identify amino acid side chains involved in receptor binding, a collection of in vitro mutagenized human BMP-2 variants was prepared and subjected to interaction analyses with use of the receptor ectodomains of BMPR-IA, BMPR-II, and ActR-II immobilized on a biosensor system. The biological activity of the BMP-2 variants was measured by BMP-2 dependent expression of alkaline phosphatase (ALP) in C2C12 cells. For crystallization, a complex of BMP-2 and the ectodomain of BMPR-IA was formed in solution, purified, and crystallized as described(12). Results: The ligand-receptor interaction analysis of the BMP-2 variants identified distinct epitopes for type I and type II receptor binding. Because the structure of TGF-.beta.-like **proteins** has been compared with that of an open hand, the binding epitope for the type I receptor was - on the basis of its location - termed "wrist" epitope. The crystal structure of the BMP-2:BMPR-IA ectodomain complex revealed a key feature of the ligand-receptor interaction: a large hydrophobic residue (Phe85) within a hydrophobic patch of BMPR-IA fit into a hydrophobic pocket composed of residues of both BMP-2 **monomers**. A second epitope identified by alanine mutagenesis scanning was termed the "knuckle" epitope on the basis of its location on the outer side of the "finger" segments of BMP-2. Mutations in either the wrist epitope or the knuckle epitope produced variants with altered biological activities. Variants with antagonistic properties were exclusively generated by mutations in the knuckle epitope of BMP-2. Conclusions and Clinical Relevance: The identification and characterization of the two receptor binding epitopes in BMP-2 provide new insight into the primary steps of BMP-receptor activation. Because of the structural similarities between members of the TGF-.beta. superfamily, it can be assumed that the data presented in this work are transferable to other TGF-.beta. receptor systems. Because of the association with various diseases, the generation of antagonists of other TGF-.beta. superfamily members might generate potent tools for basic research and therapeutic approaches.

L14 ANSWER 2 OF 3 MEDLINE DUPLICATE 1

2001163766 Document Number: 21161065. PubMed ID: 11263668. The crystal structure of the BMP-2:BMPR-IA complex and the generation of BMP-2 antagonists. Nickel J; Dreyer M K; Kirsch T; Sebald W. (Physiologische Chemie II, Biozentrum der Universtat Wurzburg, Germany.) JOURNAL OF BONE AND JOINT SURGERY. AMERICAN VOLUME, (2001) 83-A Suppl 1 (Pt 1) S7-14. Ref: 30. Journal code: HJR; 0014030. ISSN: 0021-9355. Pub. country: United States. Language: English.

AB BACKGROUND: Bone morphogenetic **proteins** (BMPs) and growth and differentiation factors (**GDFs**) belong to the large transforming growth factor-beta (TGF-beta) superfamily of multifunctional cytokines. Signaling of the BMPs requires the binding of the BMP to the BMP cell surface receptors BMPR-IA, BMPR-IB, and BMPR-II. Similar to other cytokines, members of the TGF-beta superfamily exhibit stringent specificity in their ligand-receptor interactions, which may be a reason for the qualitative and quantitative differences in cellular responses. To understand how BMPs and **GDFs** activate their receptors, it is important to determine structure and binding mechanisms of ligand-receptor complexes. We have used BMP-2 as a key representative of the BMPs to identify the epitopes for type I and type II receptor binding by mutational interaction analyses and have solved the crystal structure of a BMP2:BMPR-IA receptor ectodomain complex. METHODS: To identify amino acid side chains involved in receptor binding, a collection of in vitro mutagenized human BMP-2 variants was prepared and subjected to interaction analyses with use of the receptor ectodomains of BMPR-IA, BMPR-II, and ActR-II immobilized on a biosensor system. The biological activity of the BMP-2 variants was measured by BMP-2 dependent expression of alkaline phosphatase (ALP) in C2C12 cells. For crystallization, a complex of BMP-2

and the ectodomain of BMPR-IA was formed in solution, purified, and crystallized as described(12). RESULTS: The ligand-receptor interaction analysis of the BMP-2 variants identified distinct epitopes for type I and type II receptor binding. Because the structure of TGF-beta-like **proteins** has been compared with that of an open hand, the binding epitope for the type I receptor was-on the basis of its location-termed "wrist" epitope. The crystal structure of the BMP-2:BMPR-IA ectodomain complex revealed a key feature of the ligand-receptor interaction: a large hydrophobic residue (Phe85) within a hydrophobic patch of BMPR-IA fit into a hydrophobic pocket composed of residues of both BMP-2 **monomers** . A second epitope identified by alanine mutagenesis scanning was termed the "knuckle" epitope on the basis of its location on the outer side of the "finger" segments of BMP-2. Mutations in either the wrist epitope or the knuckle epitope produced variants with altered biological activities. Variants with antagonistic properties were exclusively generated by mutations in the knuckle epitope of BMP-2. CONCLUSIONS AND CLINICAL RELEVANCE: The identification and characterization of the two receptor binding epitopes in BMP-2 provide new insight into the primary steps of BMP-receptor activation. Because of the structural similarities between members of the TGF-beta superfamily, it can be assumed that the data presented in this work are transferable to other TGF-beta receptor systems. Because of the association with various diseases, the generation of antagonists of other TGF-beta superfamily members might generate potent tools for basic research and therapeutic approaches.

L14 ANSWER 3 OF 3 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:698246 The Genuine Article (R) Number: 352HU. Expression of a TGF-beta superfamily **protein**, macrophage inhibitory cytokine-1, in the yeast Pichia pastoris. Fairlie W D; Zhang H P; Brown P K; Russell P K; Bauskin A R; Breit S N (Reprint). ST VINCENTS HOSP, CTR IMMUNOL, SYDNEY, NSW 2010, AUSTRALIA (Reprint); ST VINCENTS HOSP, CTR IMMUNOL, SYDNEY, NSW 2010, AUSTRALIA; UNIV NEW S WALES, SYDNEY, NSW, AUSTRALIA. GENE (22 AUG 2000) Vol. 254, No. 1-2, pp. 67-76. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0378-1119. Pub. country: AUSTRALIA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB The methylotrophic yeast, Pichia pastoris, has been used to express both human and murine macrophage inhibitory cytokine-1 (MIC-1), a transforming growth factor beta (TGF-beta) superfamily cytokine. This is the first report of the expression of a correctly folded TGF-beta superfamily **protein** in a microbial organism. The **protein** is secreted in its correctly folded dimeric form at milligram per litre quantities, which are significantly higher than we have been able to achieve using mammalian expression systems. Purification schemes are described, and the purified **protein** is immunologically identical to **protein** produced in a mammalian expression system. **Protein** expression was influenced by a number of factors, most significantly by the concentration of methanol used during the induction phase. However, with very high levels of MIC-1 induction, substantial amounts of MIC-1 **monomer** were also secreted.

=> s l2 and amino acid substitution
L15 906 L2 AND AMINO ACID SUBSTITUTION

=> s l15 and TGF
L16 2 L15 AND TGF

=> dup remove l16
PROCESSING COMPLETED FOR L16
L17 2 DUP REMOVE L16 (0 DUPLICATES REMOVED)

=> d l17 1-2 cbib abs

L17 ANSWER 1 OF 2 MEDLINE

2000471997 Document Number: 20341087. PubMed ID: 10880444. BMP-2 antagonists emerge from alterations in the low-affinity binding epitope for receptor BMPR-II. Kirsch T; Nickel J; Sebald W. (Lehrstuhl fur Physiologische Chemie II, Theodor-Boveri-Institut fur Biowissenschaften (Biozentrum) der Universitat Wurzburg, Am Hubland, 97074 Wurzburg, Germany.) EMBO JOURNAL, (2000 Jul 3) 19 (13) 3314-24. Journal code: EMB; 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Bone morphogenetic **protein**-2 (BMP-2) induces bone formation and regeneration in adult vertebrates and regulates important developmental processes in all animals. BMP-2 is a homodimeric cysteine knot **protein** that, as a member of the transforming growth factor-beta (**TGF**-beta) superfamily, signals by oligomerizing type I and type II receptor serine-kinases in the cell membrane. The binding epitopes of BMP-2 for BMPR-IA (type I) and BMPR-II or ActR-II (type II) were characterized using BMP-2 mutant **proteins** for analysis of interactions with receptor ectodomains. A large epitope 1 for high-affinity BMPR-IA binding was detected spanning the interface of the BMP-2 dimer. A smaller epitope 2 for the low-affinity binding of BMPR-II was found to be assembled by determinants of a single **monomer**. Symmetry-related pairs of the two juxtaposed epitopes occur near the BMP-2 poles. Mutations in both epitopes yielded variants with reduced biological activity in C2C12 cells; however, only epitope 2 variants behaved as antagonists partially or completely inhibiting BMP-2 activity. These findings provide a framework for the molecular description of receptor recognition and activation in the BMP/**TGF**-beta superfamily.

L17 ANSWER 2 OF 2 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

92332771 EMBASE Document No.: 1992332771. Site-directed mutagenesis of glycosylation sites in the transforming growth factor-.beta.1 (**TGF** .beta.1) and **TGF**.beta.2 (414) precursors and of cysteine residues within mature **TGF**.beta.1: Effects on secretion and bioactivity. Brunner A.M.; Lioubin M.N.; Marquardt H.; Malacko A.R.; Wang W.-C.; Shapiro R.A.; Neubauer M.; Cook J.; Madisen L.; Purchio A.F.. B.-M. Squibb Pharmaceut. Res. Inst., 3005 First Avenue,Seattle, WA 98121, United States. Molecular Endocrinology 6/10 (1691-1700) 1992. ISSN: 0888-8809. CODEN: MOENEN. Pub. Country: United States. Language: English. Summary Language: English.

AB The transforming growth factor-.beta.1 (**TGF**.beta.1) and -.beta.2 (414) precursors both contain three predicted sites of N-linked glycosylation within their pro regions. These are located at amino acid residues 72, 140, and 241 for the **TGF**.beta.2 (414) precursor and at residues 82, 136, and 176 for the **TGF**.beta.1 precursor; both **proteins** contain mannose-6-phosphate (M-6-P) residues. The major sites of M-6-P addition are at Asn (82) and Asn (136), the first two sites of glycosylation, for the **TGF**.beta.1 precursor. We now show that the major site of M-6-P addition within the **TGF**.beta.2 (414) precursor is at Asn241, the third glycosylation site. To determine the importance of N-linked glycosylation to the secretion of **TGF** .beta.1 and -.beta.2, site-directed mutagenesis was used to change the Asn residues to Ser residues; the resulting DNAs were transfected into COS cells, and their supernatants were assayed for **TGF**.beta. activity. Substitution of Asn (241) of the **TGF**.beta.2 (414) precursor resulted in an 82% decrease in secreted **TGF**.beta.2 bioactivity. Mutation at Asn72 resulted in a 44% decrease, while mutation at Asn140 was without effect. Elimination of all three glycosylation sites resulted in undetectable levels of **TGF**.beta.2. These results were compared with similar mutations made in the cDNA encoding the **TGF**.beta.1 precursor. Mutagenesis of the two M-6-P-containing sites (Asn82 and Asn136) resulted in an 83% decrease in secreted **TGF**.beta.1; replacement of Asn82 and Asn136 with Ser individually resulted in 85% and 42% decreases in activity, respectively. Substitution of Asn176 with Ser was without effect, while substitution of all three sites of glycosylation resulted in undetectable levels of **TGF**

.beta.1 activity, similar to the results obtained with **TGF** .beta.2. The nine Cys residues within the mature region of **TGF** .beta.1 were mutated to serine, and their effects on **TGF**.beta.1 secretion were evaluated. Mutation of most Cys residues resulted in undetectable levels of **TGF**.beta.1 **protein** or activity in conditioned medium. Mutation of Cys (355) led to the secretion of inactive **TGF**.beta.1 **monomers**, suggesting that this residue is either directly involved in dimer formation or required for correct interchain disulfide bond formation.

```
=> s l15 and GDF
L18           0 L15 AND GDF

=> s l15 and "GDF-5"
L19           0 L15 AND "GDF-5"

=> s l15 and "MP52"
L20           0 L15 AND "MP52"

=> d his

     (FILE 'HOME' ENTERED AT 14:07:50 ON 30 MAR 2002)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 14:08:04 ON
     30 MAR 2002
L1        330132 S MONOMER
L2         57405 S L1 AND PROTEIN
L3           165 S L2 AND TGF BETA
L4             2 S L3 AND AMINO ACID SUBSTITUTION
L5             2 DUP REMOVE L4 (0 DUPLICATES REMOVED)
L6            23 S L3 AND BMP
L7             1 S L6 AND SUBSTITUTION
L8             0 S L3 AND GROWTH DIFFERENTIATION FACTOR 5
L9             4 S L3 AND GDF
L10            3 DUP REMOVE L9 (1 DUPLICATE REMOVED)
L11            3 S L2 AND GROWTH DIFFERENTIATION FACTOR
L12            3 S L2 AND GDFS
L13            4 S L2 AND GDF
L14            3 DUP REMOVE L13 (1 DUPLICATE REMOVED)
L15          906 S L2 AND AMINO ACID SUBSTITUTION
L16            2 S L15 AND TGF
L17            2 DUP REMOVE L16 (0 DUPLICATES REMOVED)
L18            0 S L15 AND GDF
L19            0 S L15 AND "GDF-5"
L20            0 S L15 AND "MP52"

=> s l15 and serine
L21           72 L15 AND SERINE

=> s l21 and threonine
L22            8 L21 AND THREONINE

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PROCESSING COMPLETED FOR L22
L23            6 DUP REMOVE L22 (2 DUPLICATES REMOVED)

=> d l23 1-6 cbib abs

L23  ANSWER 1 OF 6      MEDLINE
2001316409  Document Number: 21282866.    PubMed ID: 11388912.   Enhancement of
     the thermostability of thermophilic bacterium PS-3 PPase on substitution
     of Ser-89 with carboxylic amino acids. Wada M; Uchiumi T; Ichiba T;
     Hachimori A. (Institute of High Polymer Research, Faculty of Textile
     Science and Technology, Shinshu University, Tokida, Ueda, Nagano 386-8567,
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Japan.) JOURNAL OF BIOCHEMISTRY, (2001 Jun) 129 (6) 955-61. Journal code: HIF; 0376600. ISSN: 0021-924X. Pub. country: Japan. Language: English.

AB **Serine** 89 of the inorganic pyrophosphatase (PPase) subunit from thermophilic bacterium PS-3 (PS-3) was replaced with glycine, alanine, **threonine**, glutamic acid, or aspartic acid by the PCR-mutagenesis method with Mut-1 in order to determine the contribution of this **serine** residue to the thermostability and structural integrity of the enzyme molecule. S89G, S89A, and S89T showed reduced catalytic activity, whereas S89D and S89E showed increased enzyme activity. S89G, S89A, and S89T as well as the wild-type PPase were stable in the presence of 5 mM MgCl(2) at 70 degrees C for 1 h, but were inactivated rapidly with time at 80 degrees C. On the contrary, S89D and S89E were stable at 80 degrees C, showing more than 95% of the original activity after 1 h incubation. The wild-type PPase, S89D and S89E were each a hexamer before and after incubation at 80 degrees C for 1 h, while S89G and S89A comprised a mixture of a hexamer and a trimer both before and after incubation at 80 degrees C for 1 h. On the other hand, S89T was a mixture of a hexamer, a trimer and a **monomer**, and it was partially precipitated during heat treatment at 80 degrees C. The CD spectra of the recombinant enzymes in the far-ultraviolet region were the same as that of the wild-type PPase, whereas those of S89G, S89A, and S89T as well as the wild-type PPase were markedly different after heat treatment, although those of S89D and S89E did not change. The present study suggested that local small change(s) in the network of interactions among amino acid residues on replacement at position 89 led to the PS-3 PPase molecule being unable to form a hexamer from trimers or to dissociate into **monomers** in some cases without a significant change in the backbone conformation. It was also suggested that the partial disordering of the conformation of PS-3 PPase caused by heat depended on the degree of hydrophilicity in the vicinity of position 89.

L23 ANSWER 2 OF 6 MEDLINE

2000428462 Document Number: 20387405. PubMed ID: 10829020. Dimerization choices control the ability of axin and dishevelled to activate c-Jun N-terminal kinase/stress-activated **protein** kinase. Zhang Y; Neo S Y; Han J; Lin S C. (Regulatory Biology Laboratory, Institute of Molecular and Cell Biology, National University of Singapore, 30 Medical Drive, Singapore 117609, Republic of Singapore.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Aug 11) 275 (32) 25008-14. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Axin and Dishevelled are two downstream components of the Wnt signaling pathway. Dishevelled is a positive regulator and is placed genetically between Frizzled and glycogen synthase kinase-3beta, whereas Axin is a negative regulator that acts downstream of glycogen synthase kinase-3beta. It is intriguing that they each can activate the c-Jun N-terminal kinase/stress-activated **protein** kinase (JNK/SAPK) when expressed in the cell. We set out to address if Axin and Dishevelled are functionally cooperative, antagonistic, or entirely independent, in terms of the JNK activation event. We found that in contrast to Axin, Dvl2 activation of JNK does not require MEKK1, and complex formation between Dvl2 and Axin is independent of Axin-MEKK1 binding. Furthermore, Dvl2-DIX and Dvl2-DeltaDEP **proteins** deficient for JNK activation can attenuate Axin-activated JNK activity by disrupting Axin dimerization. However, Axin-DeltaMID, Axin-DeltaC, and Axin-CT **proteins** deficient for JNK activation cannot interfere with Dvl2-activated JNK activity. These results indicate that unlike the strict requirement of homodimerization for Axin function, Dvl2 can activate JNK either as a **monomer** or homodimer/heterodimer. We suggest that there may be a switch mechanism based on dimerization combinations, that commands cells to activate Wnt signaling or JNK activation, and to turn on specific activators of JNK in response to various environmental cues.

L23 ANSWER 3 OF 6 MEDLINE

2000471997 Document Number: 20341087. PubMed ID: 10880444. BMP-2

antagonists emerge from alterations in the low-affinity binding epitope for receptor BMPR-II. Kirsch T; Nickel J; Sebald W. (Lehrstuhl fur Physiologische Chemie II, Theodor-Boveri-Institut fur Biowissenschaften (Biozentrum) der Universitat Wurzburg, Am Hubland, 97074 Wurzburg, Germany.) EMBO JOURNAL, (2000 Jul 3) 19 (13) 3314-24. Journal code: EMB; 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Bone morphogenetic **protein**-2 (BMP-2) induces bone formation and regeneration in adult vertebrates and regulates important developmental processes in all animals. BMP-2 is a homodimeric cysteine knot **protein** that, as a member of the transforming growth factor-beta (TGF-beta) superfamily, signals by oligomerizing type I and type II receptor **serine**-kinases in the cell membrane. The binding epitopes of BMP-2 for BMPR-IA (type I) and BMPR-II or ActR-II (type II) were characterized using BMP-2 mutant **proteins** for analysis of interactions with receptor ectodomains. A large epitope 1 for high-affinity BMPR-IA binding was detected spanning the interface of the BMP-2 dimer. A smaller epitope 2 for the low-affinity binding of BMPR-II was found to be assembled by determinants of a single **monomer**. Symmetry-related pairs of the two juxtaposed epitopes occur near the BMP-2 poles. Mutations in both epitopes yielded variants with reduced biological activity in C2C12 cells; however, only epitope 2 variants behaved as antagonists partially or completely inhibiting BMP-2 activity. These findings provide a framework for the molecular description of receptor recognition and activation in the BMP/TGF-beta superfamily.

L23 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2002 ACS

1999:171334 Document No. 130:320954 Transition from monomeric to homodimeric DNA binding by nuclear receptors: identification of RevErbA.alpha. determinants required for ROR.alpha. homodimer complex formation. Moraitis, Anna N.; Giguere, Vicent (Molecular Oncology Group, McGill University Health Centre, Montreal, PQ, H3A 1A1, Can.). Mol. Endocrinol., 13(3), 431-439 (English) 1999. CODEN: MOENEN. ISSN: 0888-8809. Publisher: Endocrine Society.

AB Nuclear hormone receptors belong to a class of transcription factors that recognize specific DNA sequences either as **monomers**, homodimers, or heterodimers with the common partner retinoic X receptor. In vitro mutagenesis studies, as well as detn. of the crystal structure of several complexes formed by the DNA-binding domain of receptors bound to their cognate response elements, have begun to explain the mol. basis for **protein**-DNA and **protein-protein** interactions essential for high-affinity and specific DNA binding by nuclear receptors. In this study, we have used the related orphan nuclear receptors, ROR.alpha. and RevErbA.alpha., to study the mol. determinants involved in the transition from monomeric to homodimeric modes of DNA binding by nuclear receptors. While both receptors bind DNA as **monomers** to a response element contg. a core AGGTCA half-site preceded by a 5'-A/T-rich flanking sequence, RevErbA.alpha. also binds as a homodimer to an extended DR2 element. Gain-of-function expts. using point mutations and subdomain swaps between ROR.alpha. and RevErbA.alpha. identify four amino acids within RevErbA.alpha. sufficient to confer ROR.alpha. with the ability to form cooperative homodimer complexes on an extended DR2. This study reveals how the transition from **monomer** to homodimer DNA binding by members of the nuclear receptor superfamily could be achieved from relatively few **amino acid substitutions**

.

L23 ANSWER 5 OF 6 MEDLINE DUPLICATE 1

96260481 Document Number: 96260481. PubMed ID: 9035694. Characterization, chromosomal mapping, and expression of different ubiquitin fusion **protein** genes in tissues from control and heat-shocked maize seedlings. Liu L; Maillet D S; Frappier J R; d'Ailly K; Walden D B; Atkinson B G. (Department of Zoology, University of Western Ontario, London, Canada.) BIOCHEMISTRY AND CELL BIOLOGY, (1996) 74 (1) 9-19. Journal code: ALR; 8606068. ISSN: 0829-8211. Pub. country: Canada.

Language: English.

AB Organisms possess at least two multigene families of ubiquitins: the polyubiquitins, with few to several repeat units, which encode a ubiquitin **monomer**, and the ubiquitin fusion (or extension) **protein** genes, which encode a single ubiquitin **monomer** and a specific **protein**. This report provides details about two ubiquitin fusion **protein** genes in maize referred to as MubG7 (uwo 1) and MubG10 (uwo 2). Each has one nearly identical ubiquitin coding unit fused without an intervening nucleotide to an unrelated, 237-nucleotide sequence that encodes for a 79 amino acid **protein**. The derived amino acid sequences of the two fusion **proteins** show that they differ by five **amino acids** (**substitution** by either a **serine** or **threonine**). MubG7 maps to chromosome 8L162 and MubG10 maps to chromosome 1L131. Analyses of the role(s) of these genes in response to heat shock (1 h at 42.5 degrees C) reveal that the level of these fusion **protein** mRNAs in the radicles or plumules from 2-day-old seedlings does not change; however, heat shock does cause a marked reduction in the accumulation of these same gene-specific mRNAs in the radicles and plumules of 5-day-old seedlings. These data confirm the suggestion from our earlier work that there is precise modulation, in a gene-specific manner, of the response to developmental as well as environmental signals.

L23 ANSWER 6 OF 6 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

92241902 EMBASE Document No.: 1992241902. Fibrinogen lima: A homozygous dysfibrinogen with an A.alpha.-arginine-141 to **serine** substitution associated with extra N-glycosylation at A.alpha.-asparagine-139. Impaired fibrin gel formation but normal fibrin-facilitated plasminogen activation catalyzed by tissue-type plasminogen activator. Maekawa H.; Yamazumi K.; Muramatsu S.-I.; Kaneko M.; Hirata H.; Takahashi N.; Arocha-Pinango C.L.; Rodriguez S.; Nagy H.; Perez-Requejo J.L.; Matsuda M.. Hemostasis/Thrombosis Research Div., Institute of Hematology,Jichi Medical Tochigi-ken 329-04, Japan. Journal of Clinical Investigation 90/1 (67-76) 1992.
ISSN: 0021-9738. CODEN: JCINAO. Pub. Country: United States. Language: English. Summary Language: English.

AB An A.alpha.-arginine-141 to **serine** substitution has been identified in a homozygous dysfibrinogen, fibrinogen Lima, associated with impaired fibrin polymerization. The point mutation created an asparagine-X-**serine**-type glycosylation sequence, and indeed, extra, mainly disialylated biantennary oligosaccharides have been isolated from A.alpha. asparagine-139 of the patient's fibrinogen. This type of glycosylation sequence is unique for human fibrinogen, because the sequences shown for normal and abnormal fibrinogens are all asparagine-X-**threonine** types. The terminal sialic acids of the extra oligosaccharides seem to have largely contributed to the impaired fibrin gel formation, as evidenced by its correction to a near normal level by desialylation. Nevertheless, the polymerizing fibrin facilitated tissue-type plasminogen activator-catalyzed plasmin formation in a normal fashion, indicating that the initial two-stranded fibrin protofibrils had been constructed normally. Thus the impaired fibrin gel formation could be attributed to the delay in their subsequent lateral association, most probably because of the repulsive forces generated by the negative electric charge of the extra sialic acids. The substitution of a basic residue arginine to a noncharged residue **serine** may also have contributed to the impaired function in a similar manner or by steric hindrance in association with bulky extra oligosaccharide chains.

```
=> dup remove l21
PROCESSING COMPLETED FOR L21
L24          60 DUP REMOVE L21 (12 DUPLICATES REMOVED)

=> d l24 cbib abs
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L24 ANSWER 1 OF 60 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
2001363875 EMBASE Hierarchical **protein** folding: Asymmetric unfolding of an insulin analogue lacking the A7-B7 interchain disulfide bridge. Hua Q.-X.; Nakagawa S.H.; Jia W.; Hu S.-Q.; Chu Y.-C.; Katsoyannis P.G.; Weiss M.A.. M.A. Weiss, Department of Biochemistry, Case Western Reserve Univ. Sch. Med., Cleveland, OH 44016, United States. weiss@biochemistry.cwru.edu. Biochemistry 40/41 (12299-12311) 16 Oct 2001.
Refs: 94.
ISSN: 0006-2960. CODEN: BICHAW. Pub. Country: United States. Language: English. Summary Language: English.

AB The landscape paradigm of **protein** folding can enable preferred pathways on a funnel-like energy surface. Hierarchical preferences may be manifest as a nonrandom pathway of disulfide pairing. Stepwise stabilization of structural subdomains among on-pathway intermediates is proposed to underlie the disulfide pathway of proinsulin and related molecules. Here, effects of pairwise **serine** substitution of insulin's exposed interchain disulfide bridge (Cys(A7)-Cys(B7)) are characterized as a model of a late intermediate. Untethering cystine A7-B7 in an engineered **monomer** causes significantly more marked decreases in the thermodynamic stability and extent of folding than occur on pairwise substitution of internal cystine A6-A11 [Weiss, M. A., Hua, Q. X., Jia, W., Chu, Y. C., Wang, R. Y., and Katsoyannis, P. G. (2000) Biochemistry 39, 15429-15440]. Although substantially disordered and without significant biological activity, the untethered analogue contains a molten subdomain comprising cystine A20-B19 and a native-like cluster of hydrophobic side chains. Remarkably, A and B chains make unequal contributions to this folded moiety; the B chain retains nativelike supersecondary structure, whereas the A chain is largely disordered. These observations suggest that the B subdomain provides a template to guide folding of the A chain. Stepwise organization of insulin-like molecules supports a hierarchic view of **protein** folding.

=> s l24 and cysteine
L25 17 L24 AND CYSTEINE

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L26 ANSWER 1 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
2001200947 EMBASE Virb7 lipoprotein is exocellular and associates with the Agrobacterium tumefaciens T pilus. Sagulenko V.; Sagulenko E.; Jakubowski S.; Spudich E.; Christie P.J.. P.J. Christie, Department of Microbiology, Univ. of Texas-Houston Med. School, 6431 Fannin, Houston, TX 77030, United States. Peter.J.Christie@uth.tmc.edu. Journal of Bacteriology 183/12 (3642-3651) 2001.
Refs: 36.
ISSN: 0021-9193. CODEN: JOBAAY. Pub. Country: United States. Language: English. Summary Language: English.

AB Agrobacterium tumefaciens transfers oncogenic T-DNA and effector **proteins** to plant cells via a type IV secretion pathway. This transfer system, assembled from the products of the virB operon, is thought to consist of a transenvelope mating channel and the T pilus. When screened for the presence of VirB and VirE **proteins**, material sheared from the cell surface of octopine strain A348 was seen to possess detectable levels of VirB2 pilin, VirB5, and the VirB7 outer membrane lipoprotein. Material sheared from the cell surface of most virB gene deletion mutants also possessed VirB7, but not VirB2 or VirB5. During purification of the T pilus from wild-type cells, VirB2, VirB5, and VirB7 cofractionated through successive steps of gel filtration chromatography and sucrose density gradient centrifugation. A complex containing VirB2

and VirB7 was precipitated from a gel filtration fraction enriched for T pilus with both anti-VirB2 and anti-VirB7 antiserum. Both the exocellular and cellular forms of VirB7 migrated as disulfide-cross-linked dimers and **monomers** when samples were electrophoresed under nonreducing conditions. A mutant synthesizing VirB7 with a Ser substitution of the lipid-modified Cys15 residue failed to elaborate the T pilus, whereas a mutant synthesizing VirB7 with a Ser substitution for the disulfide-reactive Cys24 residue produced very low levels of T pilus. Together, these findings establish that the VirB7 lipoprotein localizes exocellularly, it associates with the T pilus, and both VirB7 lipid modification and disulfide cross-linking are important for T-pilus assembly. T-pilus-associated VirB2 migrated in nonreducing gels as a **monomer** and a disulfide-cross-linked homodimer, whereas cellular VirB2 migrated as a **monomer**. A strain synthesizing a VirB2 mutant with a Ser substitution for the reactive Cys64 residue elaborated T pilus but exhibited an attenuated virulence phenotype. Dithiothreitol-treated T pilus composed of native VirB2 pilin and untreated T pilus composed of the VirB2C64S mutant pilin distributed in sucrose gradients more predominantly in regions of lower sucrose density than untreated, native T pili. These findings indicate that intermolecular cross-linking of pilin **monomers** is not required for T-pilus production, but cross-linking does contribute to T-pilus stabilization.

L26 ANSWER 2 OF 17 MEDLINE

2000471997 Document Number: 20341087. PubMed ID: 10880444. BMP-2 antagonists emerge from alterations in the low-affinity binding epitope for receptor BMPR-II. Kirsch T; Nickel J; Sebald W. (Lehrstuhl fur Physiologische Chemie II, Theodor-Boveri-Institut fur Biowissenschaften (Biozentrum) der Universitat Wurzburg, Am Hubland, 97074 Wurzburg, Germany.) EMBO JOURNAL, (2000 Jul 3) 19 (13) 3314-24. Journal code: EMB; 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Bone morphogenetic **protein**-2 (BMP-2) induces bone formation and regeneration in adult vertebrates and regulates important developmental processes in all animals. BMP-2 is a homodimeric **cysteine** knot **protein** that, as a member of the transforming growth factor-beta (TGF-beta) superfamily, signals by oligomerizing type I and type II receptor **serine**-kinases in the cell membrane. The binding epitopes of BMP-2 for BMPR-IA (type I) and BMPR-II or ActR-II (type II) were characterized using BMP-2 mutant **proteins** for analysis of interactions with receptor ectodomains. A large epitope 1 for high-affinity BMPR-IA binding was detected spanning the interface of the BMP-2 dimer. A smaller epitope 2 for the low-affinity binding of BMPR-II was found to be assembled by determinants of a single **monomer**. Symmetry-related pairs of the two juxtaposed epitopes occur near the BMP-2 poles. Mutations in both epitopes yielded variants with reduced biological activity in C2C12 cells; however, only epitope 2 variants behaved as antagonists partially or completely inhibiting BMP-2 activity. These findings provide a framework for the molecular description of receptor recognition and activation in the BMP/TGF-beta superfamily.

L26 ANSWER 3 OF 17 MEDLINE

2001014288 Document Number: 20500114. PubMed ID: 11183785. Structural and biochemical features distinguish the foot-and-mouth disease virus leader proteinase from other papain-like enzymes. Guarne A; Hampoelz B; Glaser W; Carpena X; Tormo J; Fita I; Skern T. (Institut de Biologia Molecular de Barcelona, Barcelona, Spain.) JOURNAL OF MOLECULAR BIOLOGY, (2000 Oct 6) 302 (5) 1227-40. Journal code: J6V. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The structures of the two leader protease (Lpro) variants of foot-and-mouth disease virus known to date were solved using crystals in which molecules were organized as molecular fibers. Such crystals diffract to a resolution of only approximately 3 A. This singular, pseudo-polymeric organization is present in a new Lpro crystal form showing a cubic packing. As molecular fiber formation appeared unrelated to

crystallization conditions, we mutated the reactive **cysteine** 133 residue, which makes a disulfide bridge between adjacent **monomers** in the fibers, to **serine**. None of the intermolecular contacts found in the molecular fibers was present in crystals of this variant. Analysis of this Lpro structure, refined at 1.9 A resolution, enables a detailed definition of the active center of the enzyme, including the solvent organization. Assay of Lpro activity on a fluorescent hexapeptide substrate showed that Lpro, in contrast to papain, was highly sensitive to increases in the cation concentration and was active only across a narrow pH range. Examination of the Lpro structure revealed that three aspartate residues near the active site, not present in papain-like enzymes, are probably responsible for these properties.

L26 ANSWER 4 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
1999150995 EMBASE Dimerization of the calcium-sensing receptor occurs within the extracellular domain and is eliminated by Cys .fwdarw. Ser mutations at Cys101 and Cys236. Pace A.J.; Gama L.; Breitwieser G.E.. G.E. Breitwieser, Dept. of Physiology, Johns Hopkins Univ. Sch. of Medicine, 725 N. Wolfe St., Baltimore, MD 21205, United States. gbreitwi@welchlink.welch.jhu.edu. Journal of Biological Chemistry 274/17 (11629-11634) 23 Apr 1999.
Refs: 44.
ISSN: 0021-9258. CODEN: JBCHA3. Pub. Country: United States. Language: English. Summary Language: English.
AB Calcium-sensing receptors are present in membranes as dimers that can be reduced to **monomers** with sufhydryl reagents. All studies were carried out on the human calcium-sensing receptor tagged at the carboxyl terminus with green fluorescent **protein** (hCaR-GFP) to permit identification and localization of expressed **proteins**. Truncations containing either the extracellular agonist binding domain plus transmembrane helix 1 (ECD/TMH1-GFP) or the transmembrane domain plus the intracellular carboxyl terminus (TMD/carboxyl terminus-GFP) were used to identify the dimerization domain. ECD/TMH1-GFP was a dimer in the absence of reducing reagents, whereas TMD/carboxyl-terminal GFP was a **monomer** in the absence or presence of reducing agents, suggesting that dimerization occurs via the ECD. To identify the residue(s) involved in dimerization within the ECD, **cysteine** .fwdarw. **serine** point mutations were made in residues that are conserved between hCaR and metabotropic glutamate receptors. Mutations at positions 60 and 131 were expressed at levels comparable to wild type in HEK 293 cells, had minimal effects on hCaR function, and did not eliminate dimerization, whereas mutations at positions 101 and 236 greatly decreased receptor expression and resulted in significant amounts of **monomer** in the absence of reducing agents. The double point mutant hCaR(C101S/C236S)-GFP was expressed more robustly than either C101S or C236S and covalent dimerization was eliminated, hCaR(C101S/C236S)-GFP had a decreased affinity for extracellular Ca2+ and slower response kinetics upon increases or decreases in agonist concentration. These results suggest that covalent, disulfide bond-mediated dimerization of the calcium-sensing receptor contributes to stabilization of the ECD and to acceleration of the transitions between inactive and active receptor conformations.

L26 ANSWER 5 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
2000006896 EMBASE The role of **cysteine** residues in structure and enzyme activity of a maize .beta.-glucosidase. Rotrekl V.; Nejedla E.; Kucera I.; Abdallah F.; Palme K.; Brzobohaty B.. B. Brzobohaty, Institute of Biophysics AS CR, Kralovopolska 135, CZ-61265 Brno, Czech Republic. brzoboha@ibp.cz. European Journal of Biochemistry 266/3 (1056-1065) 15 Dec 1999.
Refs: 28.
ISSN: 0014-2956. CODEN: EJBCAI. Pub. Country: United Kingdom. Language: English. Summary Language: English.
AB The maize Zm-p60.1 gene encodes a .beta.-glucosidase that can release active cytokinins from their storage forms, cytokinin-O-glucosides. Mature catalytically active Zm-p60.1 is a homodimer containing five

cysteine residues per a subunit. Their role was studied by mutating them to alanine (A), **serine** (S), arginine (R) or aspartic acid (D) using site-directed mutagenesis, and subsequent heterologous expression in Escherichia coli. All substitutions of C205 and C211 resulted in decreased formation and/or stability of the homodimer, manifested as accumulation of high levels of **monomer** in the bacterial expression system. Examination of urea- and glutathione-induced dissociation patterns of the homodimer to the **monomers**, HPLC profiles of hydrolyric fragments of reduced and oxidized forms, and a homology-based three-dimensional structural model revealed that an intramolecular disulfide bridge formed between C205 and C211 within the subunits stabilized the quaternary structure of the enzyme. Mutating C52 to R produced a monomeric enzyme **protein**, too. No detectable effects on homodimer formation were apparent in C170 and C479 mutants. Given the K(m) values for C170A/S mutants were equal to that for the wild-type enzyme, C170 cannot participate in enzyme-substrate interactions. Possible indirect effects of C170A/S mutations on catalytic activity of the enzyme were inferred from slight decreases in the apparent catalytic activity, k'(cat). C170 is located on a hydrophobic side of an .alpha.-helix packed against hydrophobic aminoacid residues of .beta.-strand 4, indicating participation of C170 in stabilization of a (.beta./.alpha.)8 barrel structure in the enzyme. In C479A/D/R/S mutants, K(m) and k'(cat) were influenced more significantly suggesting a role for C479 in enzyme catalytic action.

L26 ANSWER 6 OF 17 MEDLINE

1999359373 Document Number: 99359373. PubMed ID: 10429190. The single **cysteine** residue of the Sud **protein** is required for its function as a polysulfide-sulfur transferase in Wolinella succinogenes. Klimmek O; Stein T; Pisa R; Simon J; Kroger A. (Institut fur Mikrobiologie, Johann Wolfgang Goethe-Universitat, Frankfurt am Main, Germany.) EUROPEAN JOURNAL OF BIOCHEMISTRY, (1999 Jul) 263 (1) 79-84. Journal code: EMZ; 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The periplasmic Sud **protein** which is induced in Wolinella succinogenes growing by polysulfide respiration, has been previously proposed to serve as a polysulfide binding **protein** and to transfer polysulfide-sulfur to the active site of polysulfide reductase [Klimmek, O, Kreis, V., Klein, C., Simon, J., Wittershagen, A. & Kroger, A. (1998) Eur. J. Biochem. 253, 263-269.]. The results presented in this communication suggest that polysulfide-sulfur is covalently bound to the single **cysteine** residue (Cys109) of the Sud **monomer**, and that Cys109 is required for tight binding of polysulfide-sulfur and for sulfur transfer. A modified Sud **protein** [(C109S)Sud-His6] in which the **cysteine** residue was replaced by **serine**, did not catalyze sulfur transfer from polysulfide to cyanide and did not stimulate electron transport to polysulfide, in contrast to Sud-His6. The polysulfide-sulfur bound to (C109S)Sud-His6 was fully removed upon dialysis against sulfide. After this treatment, Sud-His6 retained one sulfur atom per **monomer**; thiocyanate was formed upon addition of cyanide to the preparation. After incubation of Sud-His6 with polysulfide, a proportion of the Sud-His6 **monomers** carried one or two sulfur atoms, as shown by matrix-assisted laser desorption ionization mass spectrometry. The sulfur atoms were absent from **monomers** derived from Sud-His6 treated with cyanide and from (C109S)Sud-His6 incubated with polysulfide.

L26 ANSWER 7 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

1998317575 EMBASE Conversion of a .beta.-strand to an .alpha.-helix induced by a single-site mutation observed in the crystal structure of Fis mutant Pro26 Ala. Yang W.-Z.; Ko T.-P.; Corselli L.; Johnson R.C.; Yuan H.S.. W.-Z. Yang, Institute of Molecular Biology, Academia Sinica, Taipei 11529, Taiwan,Province of China. mbyuan@ccvax.sinica.edu.tw. Protein Science 7/9 (1875-1883) 1998.
Refs: 39.

ISSN: 0961-8368. CODEN: PRCIEI. Pub. Country: United States. Language: English. Summary Language: English.

AB The conversion from an .alpha.-helix to a .beta.-strand has received extensive attention since this structural change may induce many amyloidogenic **proteins** to self-assemble into fibrils and cause fatal diseases. Here we report the conversion of a peptide segment from a .beta.-strand to an .alpha.-helix by a single- site mutation as observed in the crystal structure of Fis mutant Pro26Ala determined at 2.0 .ANG. resolution. Pro26 in Fis occurs at the point where a flexible extended .beta.-hairpin arm leaves the core structure. Thus it can be classified as a 'hinge proline' located at the C-terminal end of the .beta.2- strand and the N-terminal cap of the A .alpha.-helix. The replacement of Pro26 to alanine extends the A .alpha.-helix for two additional turns in one of the dimeric subunits; therefore, the structure of the peptide from residues 22 to 26 is converted from a .beta.-strand to an .alpha.-helix. This result confirms the structural importance of the proline residue located at the hinge region and may explain the mutant's reduced ability to activate Hin-catalyzed DNA inversion. The peptide (residues 20 to 26) in the second **monomer** subunit presumably retains its .beta.-strand conformation in the crystal; therefore, this peptide shows a 'chameleon-like' character since it can adopt either an .alpha.- helix or a .beta.-strand structure in different environments. The structure of Pro26Ala provides an additional example where not only the **protein** sequence, but also non-local interactions determine the secondary structure of **proteins**.

L26 ANSWER 8 OF 17 MEDLINE

97327574 Document Number: 97327574. PubMed ID: 9184241. Multiple epiphyseal dysplasia and pseudoachondroplasia due to novel mutations in the calmodulin-like repeats of cartilage oligomeric matrix **protein** . Susic S; McGrory J; Ahier J; Cole W G. (Division of Orthopaedics, The Hospital for Sick Children and the University of Toronto, Ontario, Canada.) CLINICAL GENETICS, (1997 Apr) 51 (4) 219-24. Journal code: DDT; 0253664. ISSN: 0009-9163. Pub. country: Denmark. Language: English.

AB A child with a mild form of pseudoachondroplasia was heterozygous for a deletion of 12 nucleotides from exon 10 of the cartilage oligomeric matrix **protein** (COMP) gene. It resulted in the deletion of valine 513 to lysine 516 from the eighth calmodulin-like repeat of COMP **monomers** . A child with the Fairbank's type of multiple epiphyseal dysplasia was also heterozygous for a COMP mutation. It substituted **cysteine** 371 by **serine** in the fourth calmodulin-like repeat. Both mutations were likely to alter the conformation and calcium binding of the mutant COMP **protein** chains. These findings support the proposal that deletions and insertions within the calmodulin-like domain produce pseudoachondroplasia, while **amino acid** **substitutions** with this domain may produce either pseudoachondroplasia or multiple epiphyseal dysplasia.

L26 ANSWER 9 OF 17 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

1996:311506 Document No.: PREV199699033862. Site-directed mutagenesis of Cys-15 and Cys-20 of pulmonary surfactant **protein** D: Expression of a trimeric **protein** with altered anti-viral properties. Brown-Augsburger, Patricia; Hartshorn, Kevan; Chang, Donald; Rust, Kevin; Fliszar, Catherine; Welgus, Howard G.; Crouch, Edmond C. (1). (1) Dep. Pathol., Jewish Hosp. Washington Univ. Med. Cent., 216 S. Kingshighway, St. Louis, MO 63110 USA. Journal of Biological Chemistry, (1996) Vol. 271, No. 23, pp. 13724-13730. ISSN: 0021-9258. Language: English.

AB Surfactant **protein** D (SP-D) molecules are preferentially assembled as dodecamers consisting of trimeric subunits associated at their amino termini. The NH-2-terminal sequence of each **monomer** contains two conserved **cysteine** residues, which participate in interchain disulfide bonds. In order to study the roles of these residues in SP-D assembly and function, we employed site-directed mutagenesis to substitute **serine** for **cysteine** 15 and 20 in recombinant rat SP-D (RrSP-D), and have expressed the mutant

(RrSP-Dser15/20) in Chinese hamster ovary (CHO-K1) cells. The mutant, which was efficiently secreted, bound to maltosyl-agarose, but unlike RrSP-D, was assembled exclusively as trimers. The constituent **monomers** showed a decreased mobility on SDS-polyacrylamide gel electrophoresis resulting from an increase in the size and sialylation of the N-linked oligosaccharide at Asn-70. Although RrSP-Dser15/20 contained a pepsin-resistant triple helical domain, it showed a decreased T-m, and acquired susceptibility to proteolytic degradation. Like RrSP-D, RrSP-Dser15/20 bound to the hemagglutinin of influenza A. However, it showed no viral aggregation and did not enhance the binding of influenza A to neutrophils (PMN), augment PMN respiratory burst, or protect PMNs from deactivation. These studies indicate that amino-terminal disulfides are required to stabilize dodecamers, and support our hypothesis that the oligomerization of trimeric subunits contributes to the antimicrobial properties of SP-D.

L26 ANSWER 10 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
96041084 EMBASE Document No.: 1996041084. Escherichia coli RNase T functions in vivo as a dimer dependent on **cysteine** 168. Li Z.; Zhan L.; Deutscher M.P.. Dept. of Biochemistry/Molec. Biology, Univ. of Miami School of Medicine, P.O. Box 016129,Miami, FL 33101-6129, United States. Journal of Biological Chemistry 271/2 (1133-1137) 1996.
ISSN: 0021-9258. CODEN: JBCHA3. Pub. Country: United States. Language: English. Summary Language: English.
AB It was shown that Cys-168 is required for RNase T function and thermostability and that its hydrophobic properties are important for this role (Li, Z., Zhan, L., and Deutscher, M.P. (1996) J. Biol Chem. 271, 11271132). To understand the molecular basis for these findings, further studies of Cys-168 and RNase T structure were carried out. Treatment of RNase T with the sulfhydryl-modifying agent 5,5'-dithiobis-(2-nitrobenzoic acid) leads not only to inactivation, but also to monomerization of the **protein**. Similarly, specifically converting Cys-168 to either **serine** or asparagine leads to loss of activity and to **monomer** formation at 37 .degree.C. However, at 10 .degree.C the **serine** mutant remains as a dimer and retains full RNase T activity, whereas the asparagine derivative shows only a low level of activity and of dimer formation. These data show a strong correlation between activity and the dimer form of RNase T. The importance of dimer formation was also shown in vivo using genetic studies. An inactive mutant of RNase T, termed HA2, which exists as a dimer at 37 .degree.C in vitro, completely suppresses endogenous RNase T activity in vivo and in vitro when introduced into a RNase T+ cell on a multicopy phagemid, most likely as a consequence of inactive heterodimer formation. Introduction of the HA2 gene on a single-copy plasmid, as expected, leads to a proportionally smaller effect on endogenous activity. The dominant negative effect displayed by the HA2 **protein** can be relieved by an additional mutation in HA2 RNase T that abolishes its ability to dimerize. An inactive mutant asparagine derivative of Cys-168, which also does not dimerize, also shows little of the dominant negative phenotype. Thus, these data demonstrate that RNase T dimerizes in vivo, that the dimer form is required for RNase T activity, and that Cys-168 is needed for dimerization of the enzyme.

L26 ANSWER 11 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
95234912 EMBASE Document No.: 1995234912. Electrospray-ionization mass spectrometry of molecular variants of a [2Fe-2S] ferredoxin. Petillot Y.; Golinelli M.-P.; Forest E.; Meyer J.. DBMS-MEP, CENG,38054 Grenoble, France. Biochemical and Biophysical Research Communications 210/3 (686-694) 1995.
ISSN: 0006-291X. CODEN: BBRCA. Pub. Country: United States. Language: English. Summary Language: English.
AB The [2Fe-2S] ferredoxin from Clostridium pasteurianum is a homodimeric **protein** of which each subunit contains one [2Fe-2S] cluster. In previous investigations, the five **cysteine** residues in positions 11, 14, 24, 56 and 60 had been mutated into **serine** or alanine.

The wild type ferredoxin and several of its molecular variants have now been analyzed by electrospray-ionization mass spectrometry. In the negative-ion detection mode, depending on the infusion solvent used, molecular peaks attributable to the apoprotein, to the monomeric holoprotein, and to the dimeric holoprotein were detected in all cases. The data confirmed the presence of the expected mutations, showed that all of these **proteins** contain one [2Fe-2S] cluster per subunit, and indicated that the dimeric structure of these ferredoxins could be retained in the conditions of the electrospray ionization. This investigation establishes the power of electrospray-ionization mass spectrometry for the analysis of oligomeric **proteins** containing labile metal clusters.

L26 ANSWER 12 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
95007862 EMBASE Document No.: 1995007862. The stability of the molecular chaperonin cpn60 is affected by site- directed replacement of **cysteine** 518. Luo G.-X.; Horowitz P.M.. Dept. of Biochemistry, Texas Univ. Health Science Center, 7703 Floyd Curl Dr.,San Antonio, TX 78284-7760, United States. Journal of Biological Chemistry 269/51 (32151-32154) 1994.
ISSN: 0021-9258. CODEN: JBCHA3. Pub. Country: United States. Language: English. Summary Language: English.
AB **Cysteine** 518 of the molecular chaperonin cpn60 (groEL) has been replaced with **serine** (C518S) by site-directed mutagenesis. The resulting mutant chaperonin **protein** is still functional and it can: (a) arrest the spontaneous folding of rhodanese in the absence of GroES and ATP, (b) assist refolding of the enzyme rhodanese in the presence of GroES and ATP/Mg2+, and (c) permit the urea-induced release and refolding of rhodanese from its complex with cpn60. ATP/Mg2+, alone, could discharge active rhodanese from cpn60 complexes formed with either wild type or C518S. In contrast with wild type cpn60, C518S has: (a) reduced stability of its quaternary structure, (b) reduced ability to reassemble tetradecamers after dissociation by urea; (c) reduced ATPase activity; and (d) more easily exposed hydrophobic surfaces. The data suggest that replacement of Cys-518 with Ser in cpn60 destabilizes its oligomeric structure, but there is no significant effect on cpn60 function or the stability of the **monomers** formed in urea.

L26 ANSWER 13 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
94233458 EMBASE Document No.: 1994233458. Dimerization of thiol-specific antioxidant and the essential role of **cysteine** 47. Ho Zoon Chae; Tai Boong Uhm; Sue Goo Rhee. Laboratory of Biochemistry, NHLBI, National Institutes of Health,Bethesda, MD 20892, United States. Proceedings of the National Academy of Sciences of the United States of America 91/15 (7022-7026) 1994.
ISSN: 0027-8424. CODEN: PNASA6. Pub. Country: United States. Language: English. Summary Language: English.
AB Thiol-specific antioxidant (TSA) from yeast contains **cysteine** residues at amino acid positions 47 and 170 but is not associated with obvious redox cofactors. These two **cysteines** are highly conserved in a family of **proteins** that exhibit sequence identity of 23-98% with TSA. The roles of Cys-47 and Cys-170 in yeast TSA were investigated by replacing them individually with **serine** and expressing the mutant TSA **proteins** (RC47S and RC170S, respectively), as well as wild-type TSA (RWT), in Escherichia coli. Wild-type TSA purified from yeast (YWT) and RWT were both shown to exist predominantly as dimers, whereas RC47S and RC170S existed mainly as **monomers** under a denaturing condition. This observation suggests that the dimerization of YWT and RWT requires disulfide linkage of Cys-47 and Cys-170. The presence of the Cys-47-Cys-170 linkage in YWT was directly shown by isolation of dimeric tryptic peptides, one **monomer** of which contained Cys-47 and the other contained Cys-170. A small percentage of YWT, RWT, RC47S, and RC170S molecules formed dimers linked by Cys-47-Cys-47 or Cys-170-Cys-170 disulfide bonds. The antioxidant activity of the various TSA **proteins** was evaluated

from their ability to protect glutamine synthetase against the dithiothreitol/Fe3+/O2 oxidation system. YWT, RWT, and RC170S were equally protective, whereas RC47S was completely ineffective. Thus, Cys-47, but not Cys-170, constitutes the site of oxidation by putative substrate.

L26 ANSWER 14 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
92207444 EMBASE Document No.: 1992207444. Apple four in human blood coagulation factor XI mediates dimer formation. Meijers J.C.M.; Mulvihill E.R.; Davie E.W.; Chung D.W.. Department of Biochemistry, University of Washington,Seattle, WA 98195, United States. Biochemistry 31/19 (4680-4684) 1992.
ISSN: 0006-2960. CODEN: BICHAW. Pub. Country: United States. Language: English. Summary Language: English.
AB Human blood coagulation factor XI is a dimer composed of two identical subunits. Each subunit contains four apple domains as tandem repeats followed by a **serine** protease region. A disulfide bridge between Cys321 of each fourth apple domain links the subunits together. The role of Cys321 in the dimerization of factor XI was examined by mutagenesis followed by expression of its cDNA in baby hamster kidney cells. The recombinant **proteins** were then purified from the tissue culture medium and shown to have full biological activity. Normal recombinant factor XI was secreted as a dimer as determined by SDS-PAGE, while recombinant factor XI-Cys321Ser migrated as a **monomer** under these conditions. Gel filtration studies, however, revealed that each **protein** existed as a dimer under native conditions, indicating that the disulfide bond between Cys321 of each factor XI **monomer** was not necessary for dimer formation. The fourth apple domain (apple4) of factor XI was then introduced into tissue plasminogen activator (tPA) to investigate its role in the dimerization of other polypeptide chains. The fusion **protein**, containing apple4 (apple4-tPA), formed dimers as detected by SDS-PAGE and gel filtration. Furthermore, dimerization was specific to apple4, while apple3 had no effect on dimerization. These data further indicated that the apple4 domain of factor XI mediates dimerization of the two subunits and the interchain disulfide bond involving Cys321 was not essential for dimer formation.

L26 ANSWER 15 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
92332771 EMBASE Document No.: 1992332771. Site-directed mutagenesis of glycosylation sites in the transforming growth factor-.beta.1 (TGF.beta.1) and TGF.beta.2 (414) precursors and of **cysteine** residues within mature TGF.beta.1: Effects on secretion and bioactivity. Brunner A.M.; Lioubin M.N.; Marquardt H.; Malacko A.R.; Wang W.-C.; Shapiro R.A.; Neubauer M.; Cook J.; Madisen L.; Purchio A.F.. B.-M. Squibb Pharmaceut. Res. Inst., 3005 First Avenue,Seattle, WA 98121, United States. Molecular Endocrinology 6/10 (1691-1700) 1992.
ISSN: 0888-8809. CODEN: MOENEN. Pub. Country: United States. Language: English. Summary Language: English.
AB The transforming growth factor-.beta.1 (TGF.beta.1) and -.beta.2 (414) precursors both contain three predicted sites of N-linked glycosylation within their pro regions. These are located at amino acid residues 72, 140, and 241 for the TGF.beta.2 (414) precursor and at residues 82, 136, and 176 for the TGF.beta.1 precursor; both **proteins** contain mannose-6-phosphate (M-6-P) residues. The major sites of M-6-P addition are at Asn (82) and Asn (136), the first two sites of glycosylation, for the TGF.beta.1 precursor. We now show that the major site of M-6-P addition within the TGF.beta.2 (414) precursor is at Asn241, the third glycosylation site. To determine the importance of N-linked glycosylation to the secretion of TGF.beta.1 and -.beta.2, site-directed mutagenesis was used to change the Asn residues to Ser residues; the resulting DNAs were transfected into COS cells, and their supernatants were assayed for TGF.beta. activity. Substitution of Asn (241) of the TGF.beta.2 (414) precursor resulted in an 82% decrease in secreted TGF.beta.2 bioactivity. Mutation at Asn72 resulted in a 44% decrease, while mutation at Asn140 was without effect. Elimination of all three glycosylation sites resulted in undetectable levels of TGF.beta.2. These results were compared with

similar mutations made in the cDNA encoding the TGF.beta.1 precursor. Mutagenesis of the two M-6-P-containing sites (Asn82 and Asn136) resulted in an 83% decrease in secreted TGF.beta.1; replacement of Asn82 and Asn136 with Ser individually resulted in 85% and 42% decreases in activity, respectively. Substitution of Asn176 with Ser was without effect, while substitution of all three sites of glycosylation resulted in undetectable levels of TGF.beta.1 activity, similar to the results obtained with TGF.beta.2. The nine Cys residues within the mature region of TGF.beta.1 were mutated to **serine**, and their effects on TGF.beta.1 secretion were evaluated. Mutation of most Cys residues resulted in undetectable levels of TGF.beta.1 **protein** or activity in conditioned medium. Mutation of Cys (355) led to the secretion of inactive TGF.beta.1 **monomers**, suggesting that this residue is either directly involved in dimer formation or required for correct interchain disulfide bond formation.

L26 ANSWER 16 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
92255193 EMBASE Document No.: 1992255193. Detection of two missense mutations and characterization of a repeat polymorphism in the factor VII gene (F7). Marchetti G.; Patracchini P.; Gemmati D.; DeRosa V.; Pinotti M.; Rodorigo G.; Casonato A.; Girolami A.; Bernardi F.. Centro di Studi Biochimici delle, Patologie del Genoma Umano, Ist. Chimica Biol./Univ.di Ferrara, Via L,Borsari, 46, I-44100 Ferrara, Italy. Human Genetics 89/5 (497-502) 1992.
ISSN: 0340-6717. CODEN: HUGEDQ. Pub. Country: Germany. Language: English. Summary Language: English.
AB The 3' portion of the coagulation factor VII gene, containing the activation and **serine** protease domains, was investigated in four subjects with factor VII deficiency by temperature gradient gel electrophoresis and sequencing of polymerase chain reaction (PCR) products. Molecules displaying an altered melting behaviour were detected in three subjects. and direct sequencing showed two mutations. A G-to-T transversion causing a missense mutation, Cys-310 to Phe, suppresses a disulphide bond conserved in the catalytic domain of all **serine** proteases. This mutation, which in the homozygous form causes a severe reduction in protease activity (4%), was found in two patients from different Italian regions. A G-to-A transition, which gives rise to a missense mutation, Arg-304 to Gln, and is associated with the factor VII Padua variant, was found in the heterozygous form in a subject also affected by von Willebrand disease. Two polymorphic alleles, which differ in one repeat **monomer** element, were precisely mapped in a region spanning the exon-intron 7 border of the factor VII gene and studied in families with factor VII or X deficiency.

L26 ANSWER 17 OF 17 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1990:168264 Document No.: BR38:79052. SURFACTANT **PROTEIN** SP-B SPECIFIC **AMINO ACID SUBSTITUTION** AFFECT PHYSICO-CHEMICAL PROPERTIES OF PULMONARY SURFACTANT. BRUNI R; WARING A J; TAEUSCH H W. KING/DREW MED. CENT. DEP. PEDIATRICS, LOS ANGELES, CALIF.. ANNUAL MEETING OF THE WESTERN SOCIETY FOR PEDIATRIC RESEARCH, CARMEL, CALIFORNIA, USA, FEBRUARY 6-9, 1990. CLIN RES. (1990) 38 (1), 201A. CODEN: CLREAS. ISSN: 0009-9279. Language: English.

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(FILE 'HOME' ENTERED AT 14:07:50 ON 30 MAR 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 14:08:04 ON 30 MAR 2002

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L1        330132 S MONOMER
L2         57405 S L1 AND PROTEIN
L3           165 S L2 AND TGF BETA
L4             2 S L3 AND AMINO ACID SUBSTITUTION
L5             2 DUP REMOVE L4 (0 DUPLICATES REMOVED)
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L6            23 S L3 AND BMP
L7             1 S L6 AND SUBSTITUTION
L8             0 S L3 AND GROWTH DIFFERENTIATION FACTOR 5
L9             4 S L3 AND GDF
L10            3 DUP REMOVE L9 (1 DUPLICATE REMOVED)
L11            3 S L2 AND GROWTH DIFFERENTIATION FACTOR
L12            3 S L2 AND GDFS
L13            4 S L2 AND GDF
L14            3 DUP REMOVE L13 (1 DUPLICATE REMOVED)
L15          906 S L2 AND AMINO ACID SUBSTITUTION
L16            2 S L15 AND TGF
L17            2 DUP REMOVE L16 (0 DUPLICATES REMOVED)
L18            0 S L15 AND GDF
L19            0 S L15 AND "GDF-5"
L20            0 S L15 AND "MP52"
L21           72 S L15 AND SERINE
L22            8 S L21 AND THREONINE
L23            6 DUP REMOVE L22 (2 DUPLICATES REMOVED)
L24           60 DUP REMOVE L21 (12 DUPLICATES REMOVED)
L25           17 S L24 AND CYSTEINE
L26           17 DUP REMOVE L25 (0 DUPLICATES REMOVED)

=> s l15 and alanine
L27          115 L15 AND ALANINE

=> s l17 and cysteine
L28            2 L17 AND CYSTEINE

=> s l27 and alanine
L29          115 L27 AND ALANINE

=> s l29 and cysteine
L30           13 L29 AND CYSTEINE

=> dup remove l30
PROCESSING COMPLETED FOR L30
L31           13 DUP REMOVE L30 (0 DUPLICATES REMOVED)

=> d l31 1-13 cbib abs

L31  ANSWER 1 OF 13      MEDLINE
2000496044  Document Number: 20428731.    PubMed ID: 10874032.   Cryptic dimer
     interface and domain organization of the extracellular region of
     metabotropic glutamate receptor subtype 1. Tsuji Y; Shimada Y; Takeshita
     T; Kajimura N; Nomura S; Sekiyama N; Otomo J; Usukura J; Nakanishi S;
     Jingami H. (Departments of Molecular Biology and Structural Biology,
     Biomolecular Engineering Research Institute, 6-2-3 Furuedai, Suita-City,
     Osaka 565-0874, Japan. ) JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Sep 8) 275
     (36) 28144-51.  Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub.
     country: United States. Language: English.
AB   Previously, we produced the whole extracellular region of metabotropic
     glutamate receptor subtype 1 (mGluR1) in a soluble form. The soluble
     receptor retained a ligand affinity comparable with that of the
     full-length membrane-bound receptor and formed a disulfide-linked dimer.
     Here, we have identified a cysteine residue responsible for the
     intermolecular disulfide bond and determined domain organization of the
     extracellular region of mGluR1. A mutant, C140A, was a monomer
     under nonreduced conditions by SDS-polyacrylamide gel electrophoresis;
     however, C140A was eluted at the position similar to that of mGluR113, the
     wild type soluble receptor, by size exclusion column chromatography.
     Furthermore, C140A bound a ligand, [(3)H]quisqualate, with an affinity
     similar to that obtained by mGluR113. Oocytes injected with RNA for
     full-length mGluR1 containing C140A mutation showed responses to ligands
     at magnitudes similar to those with wild type full-length RNA. Thus,
     elimination of the disulfide linkage did not perturb the dimer formation
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and ligand signaling, suggesting that cryptic dimer interface(s) possibly exist in mGluR1. Limited proteolysis of the whole extracellular fragment (residue 33-592) revealed two trypsin-sensitive sites, after the residues Arg(139) and Arg(521). A 15-kDa NH(2)-terminal proteolytic fragment (residue 33-139) was associated with the downstream part after the digestion. Arg(521) was located before a **cysteine**-rich stretch preceding the transmembrane region. A new shorter soluble receptor (residue 33-522) lacking the **cysteine**-rich region was designed based on the protease-sensitive boundary. The purified receptor **protein** gave a K(d) value of 58.1 +/- 0.84 nm, which is compatible to a reported value of the full-length receptor. The B(max) value was 7.06 +/- 0. 82 nmol/mg of **protein**. These results indicated that the ligand-binding specificity of mGluR1 is confined to the NH(2)-terminal 490-amino acid region of the mature **protein**.

L31 ANSWER 2 OF 13 MEDLINE

2001132524 Document Number: 21040477. PubMed ID: 11200939. Human chorionic gonadotropin beta-subunit affects the folding and glycosylation of alpha-cys mutants. Furuhashi M; Suganuma N. (Department of Obstetrics and Gynecology, Handa City Hospital, Handa, Aichi, Japan.) ENDOCRINE JOURNAL, (2000 Oct) 47 (5) 583-9. Journal code: BT5; 9313485. ISSN: 0918-8959. Pub. country: Japan. Language: English.

AB Human chorionic gonadotropin (hCG) is a member of a family of heterodimeric glycoprotein hormones that contain a common alpha-subunit but differ in their hormone-specific beta-subunits. Both subunits have five and six disulfide bonds, respectively, which consist of cystine knot structure. It is evident from numerous studies that the structure of beta-subunits is rigid, whereas that of alpha-subunit is flexible and can be molded by a beta-subunit. Previously, we reported that secreted forms of a mutants where either **cysteine** residue in the disulfide bond 7-31 or 59-87 was converted to **alanine** contained a disulfide-linked homodimer in addition to a **monomer**. To study whether the hCGbeta-subunit affects the conformations of alpha mutants, alpha-subunits lacking either the 7-31 or 59-87 disulfide bond were expressed with wild-type (WT) hCGbeta in Chinese hamster ovary cells, and homodimer formation and glycosylation of dimerized alpha-subunit were assessed by continuous labeling with [35S]methionine/**cysteine**, immunoprecipitation with anti-alpha or -hCGbeta serum, digestion with endoglycosidase-H or -F, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a non-reducing condition. Our data showed that a homodimer was not observed in the half-Cys mutants except one, where **cysteine** at position 7 was converted to **alanine**, in the presence of beta-subunit. This finding indicated that hCGbeta-subunit rescued the a half-Cys mutants from the formation of intermolecular disulfide-linked homodimer by preferentially combining with the alpha mutants. In both free WT and all mutants treated with endoglycosidase-H, no or faint bands were recognized as the same migration as seen in endoglycosidase-F treatment. Even in the endoglycosidase-H sensitive cases, the amount of sensitive alpha-subunits was less than 5% of total alpha-subunits. In contrast to free alpha-subunits, distinct endoglycosidase-H sensitive bands were seen in both WT and mutants, although the ratio was various. We concluded that hCGbeta-subunit affects the folding and glycosylation of the alpha-subunit mutants.

L31 ANSWER 3 OF 13 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

2000006896 EMBASE The role of **cysteine** residues in structure and enzyme activity of a maize .beta.-glucosidase. Rotrekl V.; Nejedla E.; Kucera I.; Abdallah F.; Palme K.; Brzobohaty B.. B. Brzobohaty, Institute of Biophysics AS CR, Kralovopolska 135, CZ-61265 Brno, Czech Republic. brzoboha@ibp.cz. European Journal of Biochemistry 266/3 (1056-1065) 15 Dec 1999.
Refs: 28.
ISSN: 0014-2956. CODEN: EJBCAI. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB The maize Zm-p60.1 gene encodes a .beta.-glucosidase that can release

active cytokinins from their storage forms, cytokinin-O-glucosides. Mature catalytically active Zm-p60.1 is a homodimer containing five **cysteine** residues per a subunit. Their role was studied by mutating them to **alanine** (A), serine (S), arginine (R) or aspartic acid (D) using site-directed mutagenesis, and subsequent heterologous expression in Escherichia coli. All substitutions of C205 and C211 resulted in decreased formation and/or stability of the homodimer, manifested as accumulation of high levels of **monomer** in the bacterial expression system. Examination of urea- and glutathione-induced dissociation patterns of the homodimer to the **monomers**, HPLC profiles of hydrolyric fragments of reduced and oxidized forms, and a homology-based three-dimensional structural model revealed that an intramolecular disulfide bridge formed between C205 and C211 within the subunits stabilized the quaternary structure of the enzyme. Mutating C52 to R produced a monomeric enzyme **protein**, too. No detectable effects on homodimer formation were apparent in C170 and C479 mutants. Given the K(m) values for C170A/S mutants were equal to that for the wild-type enzyme, C170 cannot participate in enzyme-substrate interactions. Possible indirect effects of C170A/S mutations on catalytic activity of the enzyme were inferred from slight decreases in the apparent catalytic activity, k'(cat). C170 is located on a hydrophobic side of an .alpha.-helix packed against hydrophobic aminoacid residues of .beta.-strand 4, indicating participation of C170 in stabilization of a (.beta./.alpha.)8 barrel structure in the enzyme. In C479A/D/R/S mutants, K(m) and k'(cat) were influenced more significantly suggesting a role for C479 in enzyme catalytic action.

L31 ANSWER 4 OF 13 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

1999094574 EMBASE Mapping of structural determinants for the oligomerization of p58, a lectin-like **protein** of the intermediate compartment and cis-Golgi. Lahtinen U.; Svensson K.; Pettersson R.E.. R.E. Pettersson, Ludwig Institute for Cancer Research, Box 240, S-17177 Stockholm, Sweden. rpet@licr.ki.se. European Journal of Biochemistry 260/2 (392-397) 1 Mar 1999.

Refs: 37.

ISSN: 0014-2956. CODEN: EJBCAI. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB Shortly after synthesis, p58, the rat homologue of the mannose-binding lectin ERGIC-53/MR60, which localizes to pre-Golgi and cis-Golgi compartments, forms dimers and hexamers, after which an equilibrium of both forms is reached. Mature p58, a type I membrane **protein**, contains four **cysteine** residues in the lumenal domain which are capable of forming disulphide bonds. The membrane-proximal half of the lumenal domain consists of four predicted .alpha.-helical domains, one heavily charged and three amphipathic in nature, all candidates for electrostatic or coiled-coil interactions. Using single-stranded mutagenesis, the **cysteines** were individually changed to **alanines** and the contribution of each of the .alpha.- helical domains was probed by internal deletions. The N-terminal **cysteine** to **alanine** mutants, C198A and C238A and the double mutant, C198/238A, oligomerized like the wild-type **protein**. The two membrane-proximal **cysteines** were found to be necessary for the oligomerization of p58. Mutants lacking one of the membrane proximal **cysteines**, either C473A or C482A, were unable to form hexamers, while dimers were formed normally. The C473/482A double mutant formed only **monomers**. Deletion of any of the individual .alpha.-helical domains had no effect on oligomerization. The dimeric and hexameric forms bound equally well to D-mannose. The dimeric and monomeric mutants displayed a cellular distribution similar to the wild-type **protein** , indicating that the oligomerization status played a minimal role in maintaining the subcellular distribution of p58.

L31 ANSWER 5 OF 13 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

1998317575 EMBASE Conversion of a .beta.-strand to an .alpha.-helix induced by a single-site mutation observed in the crystal structure of Fis mutant

Pro26 Ala. Yang W.-Z.; Ko T.-P.; Corselli L.; Johnson R.C.; Yuan H.S.. W.-Z. Yang, Institute of Molecular Biology, Academia Sinica, Taipei 11529, Taiwan,Province of China. mbyuan@ccvax.sinica.edu.tw. Protein Science 7/9 (1875-1883) 1998.
Refs: 39.
ISSN: 0961-8368. CODEN: PRCIEI. Pub. Country: United States. Language: English. Summary Language: English.

AB The conversion from an .alpha.-helix to a .beta.-strand has received extensive attention since this structural change may induce many amyloidogenic **proteins** to self-assemble into fibrils and cause fatal diseases. Here we report the conversion of a peptide segment from a .beta.-strand to an .alpha.-helix by a single- site mutation as observed in the crystal structure of Fis mutant Pro26Ala determined at 2.0 .ANG. resolution. Pro26 in Fis occurs at the point where a flexible extended .beta.-hairpin arm leaves the core structure. Thus it can be classified as a 'hinge proline' located at the C-terminal end of the .beta.2- strand and the N-terminal cap of the A .alpha.-helix. The replacement of Pro26 to **alanine** extends the A .alpha.-helix for two additional turns in one of the dimeric subunits; therefore, the structure of the peptide from residues 22 to 26 is converted from a .beta.-strand to an .alpha.-helix. This result confirms the structural importance of the proline residue located at the hinge region and may explain the mutant's reduced ability to activate Hin-catalyzed DNA inversion. The peptide (residues 20 to 26) in the second **monomer** subunit presumably retains its .beta.-strand conformation in the crystal; therefore, this peptide shows a 'chameleon-like' character since it can adopt either an .alpha.- helix or a .beta.-strand structure in different environments. The structure of Pro26Ala provides an additional example where not only the **protein** sequence, but also non-local interactions determine the secondary structure of **proteins**.

L31 ANSWER 6 OF 13 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
1998042969 EMBASE Specificity of RNA binding by CPEB: Requirement for RNA recognition motifs and a novel zinc finger. Hake L.E.; Mendez R.; Richter J.D.. J.D. Richter, Dept. of Molec. Genetics/Microbiol., Univ. of Massachusetts Med. School, 222 Maple Ave., Shrewsbury, MA 01545, United States. joel.richter@banyan.ummed.edu. Molecular and Cellular Biology 18/2 (685-693) 1998.
Refs: 43.
ISSN: 0270-7306. CODEN: MCEBD4. Pub. Country: United States. Language: English. Summary Language: English.

AB CPEB is an RNA binding **protein** that interacts with the maturation-type cytoplasmic polyadenylation element (CPE) (consensus UUUUUAU) to promote polyadenylation and translational activation of maternal mRNAs in Xenopus laevis. CPEB, which is conserved from mammals to invertebrates, is composed of three regions: an amino-terminal portion with no obvious functional motif, two RNA recognition motifs (RRMs), and a **cysteine**-histidine region that is reminiscent of a zinc finger. In this study, we investigated the physical properties of CPEB required for RNA binding. CPEB can interact with RNA as a **monomer**, and phosphorylation, which modifies the **protein** during oocyte maturation, has little effect on RNA binding. Deletion mutations of CPEB have been overexpressed in Escherichia coli and used in a series of RNA gel shift experiments. Although a full-length and a truncated CPEB that lacks 139 amino-terminal amino acids bind CPE-containing RNA avidly, **proteins** that have had either RRM deleted bind RNA much less efficiently. CPEB that has had the **cysteine**-histidine region deleted has no detectable capacity to bind RNA. Single **alanine** substitutions of specific **cysteine** or histidine residues within this region also abolish RNA binding, pointing to the importance of this highly conserved domain of the **protein**. Chelation of metal ions by 1,10- phenauthroline inhibits the ability of CPEB to bind RNA; however, RNA binding is restored if the reaction is supplemented with zinc. CPEB also binds other metals such as cobalt and cadmium, but these destroy RNA binding. These data indicate that the RRMs and a zinc finger region of

CPEB are essential for RNA binding.

L31 ANSWER 7 OF 13 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
1998082956 EMBASE The dimerization of Pseudomonas putida cytochrome P450(cam): Practical consequences and engineering of a monomeric enzyme. Nickerson D.P.; Wong L.-L.. L.-L. Wong, Department of Chemistry, Inorganic Chemistry Laboratory, South Parks Road, Oxford OX1 3QR, United Kingdom. Protein Engineering 10/12 (1357-1361) 1997.
Refs: 25.
ISSN: 0269-2139. CODEN: PRENE. Pub. Country: United Kingdom. Language: English. Summary Language: English.
AB Cytochrome P450(cam) dimerizes via the formation of an intermolecular disulfide bond, complicating the storage and handling of the enzyme, particularly at higher concentrations. The dimeric enzyme is 14% less active than the **monomer** and forms at a slow but significant rate even at 4.degree.C (k = 1.09 x 10-3 mM-1 h-1). To eliminate any ambiguity introduced by dimer formation and to simplify handling and storage of the enzyme, site-directed mutagenesis was used to identify C334 as the single **cysteine** residue responsible for the formation of the disulfide linkage and to engineer a monomeric enzyme by substituting an **alanine** in its place. The C334A mutant is identical with the wild-type P450(cam) **monomer** in terms of optical spectra, camphor binding and turnover activity, but shows no evidence of dimerization and aggregation even at millimolar concentrations. Preliminary 1H NMR investigations also indicate a significant improvement in the quality of spectra obtained with this mutant. (C334A)P450(cam) is therefore proposed as an alternative to the wild-type enzyme - a base mutant otherwise identical with the wild-type but with improved handling characteristics.

L31 ANSWER 8 OF 13 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
95234912 EMBASE Document No.: 1995234912. Electrospray-ionization mass spectrometry of molecular variants of a [2Fe-2S] ferredoxin. Petillot Y.; Golinelli M.-P.; Forest E.; Meyer J.. DBMS-MEP, CENG,38054 Grenoble, France. Biochemical and Biophysical Research Communications 210/3 (686-694) 1995.
ISSN: 0006-291X. CODEN: BBRCA. Pub. Country: United States. Language: English. Summary Language: English.
AB The [2Fe-2S] ferredoxin from Clostridium pasteurianum is a homodimeric **protein** of which each subunit contains one [2Fe-2S] cluster. In previous investigations, the five **cysteine** residues in positions 11, 14, 24, 56 and 60 had been mutated into serine or **alanine**. The wild type ferredoxin and several of its molecular variants have now been analyzed by electrospray-ionization mass spectrometry. In the negative-ion detection mode, depending on the infusion solvent used, molecular peaks attributable to the apoprotein, to the monomeric holoprotein, and to the dimeric holoprotein were detected in all cases. The data confirmed the presence of the expected mutations, showed that all of these **proteins** contain one [2Fe-2S] cluster per subunit, and indicated that the dimeric structure of these ferredoxins could be retained in the conditions of the electrospray ionization. This investigation establishes the power of electrospray-ionization mass spectrometry for the analysis of oligomeric **proteins** containing labile metal clusters.

L31 ANSWER 9 OF 13 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
94257525 EMBASE Document No.: 1994257525. Mutational analysis of secondary structure in the autoinhibitory and autophosphorylation domains of calmodulin kinase II. Mukherji S.; Brickey D.A.; Soderling T.R.. Vollum Institute, Oregon Health Sciences University, 3181 S.W. Sam Jackson Park Rd.,Portland, OR 97201, United States. Journal of Biological Chemistry 269/32 (20733-20738) 1994.
ISSN: 0021-9258. CODEN: JBCHA3. Pub. Country: United States. Language: English. Summary Language: English.
AB A previous study has suggested that the autoinhibitory domain of Ca2+/calmodulin-dependent **protein** kinase II (CaM-kinase II) may

contain an .alpha.-helical structure, which is important for the proper orientation of inhibitory residues to interact with the catalytic domain (Smith, M. K., Colbran, R. J., Brickey, D. A., and Soderling, T. R. (1992) J. Biol. Chem. 267, 1761-1768). The present study was designed to test the importance of the secondary structure in the autoinhibitory domain (residues 281-302) by site- specific mutagenesis of selected residues to prolines. Single mutants C289P, C289A, A295P, and the double mutant C289P/A295P were expressed using the baculovirus/Sf9 cell system and purified on CaM-Sepharose. The single mutants had specific activities (7-12 .mu.mol/min/mg) and eluted from gel permeation chromatography (600-650 kDa) identical to wild-type kinase. Since the double mutant had a very low specific activity and eluted as a mixture of a large aggregate and proteolyzed **monomer**, it was not characterized further. Only the C289P mutant exhibited enhanced Ca2+-independent activity (5-12% of total activity) prior to autophosphorylation. When autophosphorylation was performed in the absence of Ca2+/CaM at 5.degree.C, only the C289P mutant showed a significant increase in Ca2+-independent activity. This autophosphorylation of Thr286 in the absence of Ca2+/CaM has not been observed with wild-type kinase or any other autoinhibitory domain mutant we have characterized. This result suggests that Thr286, the autophosphorylation site responsible for Ca2+-independent activity, may not be available for autophosphorylation in the wild-type kinase or the other mutants because of structural restrictions due to the secondary structure in this region. This structural restraint is presumably disrupted by the binding of Ca2+/CaM or by insertion of a proline residue.

L31 ANSWER 10 OF 13 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
94112894 EMBASE Document No.: 1994112894. Functional analysis of the **cysteine** residues of activin A. Mason A.J.. Prince Henry's Med. Research Inst., P.O. Box 152,Clayton, Vic. 3168, Australia. Molecular Endocrinology 8/3 (325-332) 1994.
ISSN: 0888-8809. CODEN: MOENEN. Pub. Country: United States. Language: English. Summary Language: English.

AB Site-directed mutagenesis and mammalian cell expression was used to analyze the function of each of the 13 **cysteine** residues in the human activin A .beta.-subunit precursor. Substitution of the four **cysteine** residues in the proregion with **alanine** residues did not affect the function of the proregion in facilitating the dimerization and secretion of activin A homodimers. A series of activin mutants were constructed in which the nine **cysteine** residues (amino acids 4, 11, 12, 40, 44, 80, 81, 113, and 115) in the mature 116-amino acid .beta.-subunit were individually altered to **alanine** residues. **Alanine** substitution at either **cysteine** residues 4 or 12 did not interfere with homodimer formation, but the mutant activin A molecules had reduced biological and receptor binding activity (2- to 3-fold). Activin A **monomers** were produced when **cysteine** mutants 44, 80, and 113 were expressed in tissue culture cells. **Monomers** of cys mutants 44 and 80 had approximately 2% of the biological and receptor binding activity of wild type activin A. Cys 113 **monomers** had undetectable levels of biological activity. No detectable activin **monomers** or dimers were secreted from cells transfected with plasmids containing cys mutants 11, 40, 81, and 115. The data presented here suggest that a low level of noncovalent dimer formation of **cysteine** mutant **monomers** 44 and 80 may explain their low level of biological activity. Therefore, dimer formation is suggested to be an essential prerequisite for high affinity receptor binding and biological potency. In summary, all of the nine **cysteine** residues in the mature activin A .beta.-subunit have been shown to be essential for either the biosynthesis of activin A or the full biological activity of the molecule.

L31 ANSWER 11 OF 13 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
93175701 EMBASE Document No.: 1993175701. Intersubunit location of the active site of mammalian ornithine decarboxylase as determined by hybridization of site-directed mutants. Tobias K.E.; Kahana C.. Dept. of Molecular

Genetics/Virology, Weizmann Institute of Science,Rehovot 76100, Israel.
Biochemistry 32/22 (5842-5847) 1993.
ISSN: 0006-2960. CODEN: BICHAW. Pub. Country: United States. Language: English. Summary Language: English.

AB The active form of mammalian ornithine decarboxylase (ODC) is a homodimer consisting of two **monomer** subunits of 53 kDa each. We have used in vitro hybridization of two different catalytically inactive mutants of ODC to determine whether in the wild-type enzyme each **monomer** contains an independent active site or whether the active sites are shared at the interfaces between the two subunits. Two distinct mutants were obtained using oligonucleotide-directed mutagenesis: In one, **cysteine**-360, the major .alpha.- (difluoromethyl)ornithine (.alpha.-DFMO, a suicide inhibitor of ODC) binding site was converted to **alanine**. In the other, lysine-69, the pyridoxal 5'-phosphate (PLP, the cofactor of ODC) binding residue was converted to **alanine**. Expression of each mutant, in vitro, in reticulocyte lysate translation mix, results in the production of a completely inactive enzyme. In contrast, their coexpression restores enzymatic activity to about 25% of the wild-type enzyme. Moreover, coexpression of wild-type subunits with **monomers** containing both inactivating mutations reduced their activity to about 25%, while their coexpression with **monomers** that contain a single inactivating mutation reduced the activity to 50%. Cross-linking analysis has demonstrated that activity restoration and repression are both fully correlated with the formation of heterodimers between mutant subunits and between mutant and wild-type subunits, respectively. We therefore conclude that the active site of ODC is formed at the interface of the two **monomers** through the interaction of the **cysteine**-360-containing region of one **monomer** subunit with the region that contains lysine-69 of the other subunit.

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93021302 EMBASE Document No.: 1993021302. Interrelations between assembly and secretion of recombinant human acetylcholinesterase. Kerem A.; Kronman C.; Bar-Nun S.; Shafferman A.; Velan B.. Israel Inst. of Biological Research, P. O. Box 19,Ness-Ziona 70450, Israel. Journal of Biological Chemistry 268/1 (180-184) 1993.
ISSN: 0021-9258. CODEN: JBCHA3. Pub. Country: United States. Language: English. Summary Language: English.

AB Transport and secretion of recombinant human acetylcholinesterase (rHuAChE) were studied in transfected human 293 cells expressing either the oligomerized soluble enzyme or a monomeric mutant derivative in which Cys- 580 was substituted by **alanine** (C580A). In cells expressing the wild-type enzyme, the gradual assembly of newly synthesized intracellular rHuAChE **monomers** into oligomers occurs within the endoplasmic reticulum. Secretion of mature wild-type enzyme into the medium is efficient and appears to be exclusive to multimeric forms. Consistently, intracellular oligomers, but not **monomers**, are endoglycosidase H-resistant, indicating that only oligomers undergo terminal glycosylation in the wild-type enzyme. In contrast, in cells expressing the dimerization-defective C580A mutant, newly synthesized rHuAChE **monomers** undergo terminal glycosylation and are secreted into the medium as efficiently as wild-type multimers. No significant difference between the intracellular transport rates of wild-type rHuAChE oligomers and mutant C580A **monomers** was revealed by probing with specific lectins. In both systems, transport and processing prior to the trans-Golgi galactosylation compartment appear to be rate-limiting, whereas the following passage to the cell surface is rapid. In conclusion, we suggest that in the presence of a free **cysteine** at the COOH terminus of the rHuAChE polypeptide, secretion of **monomers** is not effectuated, whereas in its absence, **monomers** are exported from the endoplasmic reticulum and are capable of traversing the entire secretory pathway.

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1990:168264 Document No.: BR38:79052. SURFACTANT **PROTEIN** SP-B

SPECIFIC **AMINO ACID SUBSTITUTION** AFFECT
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---Logging off of STN---

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DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-0.62	-0.62

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